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NEW POLYMERS FOR PHASE PARTITIONING
(September 1, 1980 to August 31, 1981)

ANNUAL REPORT
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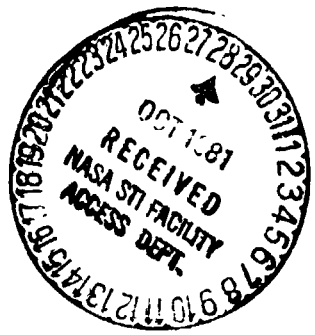

September 10, 1981

NEW POLYMERS FOR PHASE PARTITIONING - ANNUAL REPORT

ABSTRACT

During the past year we have achieved our goal of synthesizing several polyethylene glycols having crown ethers attached. Testing of these polymers in red blood cell separations will begin soon. This preliminary work has led to the identification of three new polymer types which promise to be more effective at selectively binding specific cell types; these polymers are being synthesized. Work has been completed on identification of chemical properties of the new polymer-crowns and on development of new techniques for determination of polymer-phase composition. Examination of the Ito devices for rapid phase-partitioning studies is well advanced; further work is this area awaits repair of the instrument at Marshall.

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I. WORK PERFORMED

A. Polymer Synthesis

During the past year we have achieved our goal of synthesizing several polyethylene glycols having crown ethers attached. The compounds were prepared by use of reactions 1 and 2 or 3 and 4, where TsCl is p-toluenesulfonyl chloride, the crown alcohol is either hydroxymethyl-16-crown-5 or hydroxymethyl-19-crown-6, the crown amine is diaza-18-crown-6, and PEG is polyethylene glycol.

$$\text{PEG-OH} + \text{TsCl} \xrightarrow[\text{benzene}]{\text{NaH}} \text{PEG-OTs} \qquad (1)$$

$$\text{PEG-OTs} + \text{crown-OH} \xrightarrow[\text{benzene}]{\text{NaH}} \text{PEG-crown} \qquad (2)$$

$$\text{PEG-OH} + \text{CrO}_3\text{Cl}(\text{C}_6\text{H}_5\text{N}) \xrightarrow{\text{CH}_2\text{Cl}_2} \text{PEG-CHO} \qquad (3)$$

$$\text{PEG-CHO} + \text{crown amine} + \text{NaBH}_3\text{CN} \longrightarrow \text{PEG-crown} \qquad (4)$$

The crown alcohols were synthesized by reaction 5. All other compounds were purchased.

$$\text{HOCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{OH} + (\text{ClCH}_2)_2\text{CCH}_2 \xrightarrow[(2)\ \text{B}_2\text{H}_6]{(1)\ \text{NaH}} \text{crown-OH} \qquad (5)$$

A great deal of experimentation was required to determine the proper conditions for each of these reactions. With the exception of reaction 5 none of these reactions had been done before. In the case of reaction 5 the published report neglected to give sufficient details to permit ready completion of this synthesis. So, in effect, all of the chemistry was new.

The polyethylene glycols used were obtained from Union Carbide and were of molecular weights 3400 g/mol and 6800 g/mol. Determination of polymer yields was done by chemical analysis and by molecular-weight determination by size-exclusion chromatography. This use of size-exclusion chromatography was new and was made possible by Varian's development of the necessary chromatography columns for water-soluble polymers.

B. Chemical Properties of the New Polymers

Before beginning phase partitioning of cells it is important to determine some of the chemical properties of the new polymers. The prime property in which we are interested is the ability of these materials to bind metals. This property can be examined by studying the ability of the polymers to act as phase transfer catalysts. A phase transfer catalyst acts by removing a metal cation and its accompanying anion from water into an immiscible organic liquid layer. Once the anion is in the organic liquid it is very poorly solvated, and as a consequence much more reactive. We have determined the abilities of our PEG-crowns and PEG itself to catalyze the displacement of chloride from benzyl chloride by potassium acetate in acetonitrile. The acetate ion normally has only very slight solubility in acetonitrile, so this reaction is normally very slow (a few percent reaction after 24 hours).

We found that the crown polymers are very effective phase transfer agents. More interesting was the observation that the PEG's are also effective agents, although not quite as powerful as the crown-polymers. This fact is very important in guiding our future cell partitioning experiments, since it indicates that the metal-complexing ability of the crowns will be reduced because of the competition from polymer chains.

C. Techniques For Determination of Polymer-Phase Composition

We have done preliminary experiments with the conventional dextran-PEG phase system for cell purification (see next section). In the course of this work we discovered that the conventional techniques for analyzing the composition of the phase systems was woefully deficient. The problem is three-fold. First, it is very difficult to dry dextran, so that stock solutions are made from material containing an unknown amount of water. Second, the usual technique for measuring the amount of polymers in mixtures of PEG and dextran is to determine the dextran by polarimetry, freeze-dry the solution, and determine the PEG by weight difference. This technique is tedious and inaccurate. Third, and most problematic, phase partitioning results can vary dramatically for phase systems which appear to have the same composition. This results, we believe, because of either trace amounts of low-molecular-weight impurities or variations in molecular weight of the polymers.

The three problems have been solved by application of the techniques of refractometry, polarimetry, size-exclusion chromatography (on the high performance liquid chromatograph), and gas chromatography. Refractometry provides a very sensitive measure of the concentrations of PEG or dextran in a pure aqueous solution. As was previously known, dextran concentration can be determined by polarimetry. To determine the concentrations of a PEG-dextran mixture it is necessary only to determine the dextran concentration by polarimetry, subtract the dextran contribution to the solution refractive index, and calculate the PEG concentration from the resulting calculated refractive index. This technique is very useful and easily applied on a routine basis.

An alternative technique, which is actually more revealing, is size-exclusion chromatography. This technique not only gives the concentration

of PEG and dextran, but it also provides a molecular weight profile of the polymers; it is this last property that contributes to variations in phase partitioning which occur despite constancy of phase composition (the third problem mentioned above). This technique is a little more complicated to apply than refractometry-polarimetry, and thus is not as useful on a routine basis. Finally, we have found gas chromatography to be a useful technique for detecting the presence of volatile impurities in the polymers. These impurities can also contribute to variation in phase partitioning results.

As a result of this work, we can now carefully monitor the composition and purity of our polymer solutions. This should aid greatly in the performance of the second portion of our work, the determination of the utility of the new polymers for phase partitioning.

D. Examination of the Ito Device

Dr. Y. Ito (of NIH) has developed a new device permitting rapid multiple phase partitioning experiments. Dr. Ito loaned two of these devices to Dr. Robert Synder's laboratory. We decided to spend some time experimenting with the new devices to see if they would facilitate the study of our new polymers. After much experimentation with the untested machinery we were able to reproduce some early experiments of Ito's and to gain some understanding of the operational principles of the instruments. One device, the nonsynchronous coil planet centrifuge, was deemed suitable for cell separations. Unfortunately, the instrument was not very durable, and will not function at present because of several expensive mechanical failures (e.g., the drive motor). We do now have sufficient experience on the device to permit its application to testing of our new polymers should the instrument be repaired.

II. WORK TO BE PERFORMED

The major goal of our work is to develop new polymers for use in phase-partitioning cell purifications. Now that we have some of these polymer-crowns in hand, our next task will be to examine the polymers in our proposed test system, the separation of red blood cells.

In the course of this work it has become obvious that we now have the chemical techniques available to prepare three new polymers having even more likelyhood than the PEG-crowns of introducing selectivity into the partitioning process; we also plan to synthesize these polymers. The first polymer is that resulting from binding antibodies to PEG. The antibodies will bind specific groups on cell surfaces. The econd polymer is made by binding cyclodextrins to PEG. The cyclodextrins are similar to the crown ethers, but other experiments indicate that they are more likely to bind the negative binding groups on the cell surface. The third polymer is made by attaching long hydrocarbon tails (e.g., eighteen carbons) to the PEG by an ether linkage. Experiments in Don Brook's laboratory have shown that these tails can be very selective for binding certain cell types. Previously, however, the tails have been attached by ester linkages and have proven to be unstable. We believe that attachment via an ether linkage will give a stable and thus much more useful material. These three polymer types will be examined in red blood cell separations.

III. CURRENT PROBLEMS

At present the only problem which might impede the performance of our work is the availability of the Ito device. We can conduct our experiments without this instrument, but its availability could speed our testing process.

FINANCIAL STATUS REPORT

Contract No. NAS8-33978

Period: 9/1/80 - 8/31/81

1.	Contract Value	$93,716.00 *
2.	1st increment allotted	46,000.00
3.	Expenditures to date	43,003.67
4.	Estimated Funds to completion	926.20
5.	Anticipated over/under run	
6.	Changes authorized but not finalized	
7.	Changes under consideration but not authorized	

*$47,716 still to be allotted